

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

114

11/30/94

From Page No. \_\_\_\_\_

Prepared: Amplify BARDH for ~~and~~ ~~different~~ - cloning and ~~different~~ in different enzymes again.

- BARDH / Regular Forward + Reverse primers / DeepVent worked (pg 97)

- never tried with Tag + DeepVent,

- but no problem with Tag alone (pg 88).

used: DeepVent buffer - 2 mM Mg  
200 μM dNTP  
0.15 μM primer  
100 pg template (10 pg/λ)

95° 5'  
25 (94°, 30", 60°, 1' 15"  
72°, 10'

Tag:	Unit:	Tag + DeepVent	DeepVent
# 1 2	0	# 31 32 10 + .001	# 15 16
3 4	.5	33 34 .005	17 18
5 6	1	35 36 .01	19 20
7 8	1.5	37 38 .05	21 22
9 10	2	39 40 .1	23 24
11 12	2.5	41 42 .2	25 26
13 14	5	43 44 .5	27 28
15		45 46 1	45 46
15x		17x	47 48

10x buffer 75  
dNTP 15  
Template 150  
primer 1 3.75  
2 3.75

50 μl / Rx

Cocktail → 50 μl / Rx

enzyme diff amount  
D.V. + 10 Tag in all

20x  
50 μl  
+  
diff. am  
2  
deep vent

added enzyme  
separately in 1 μl

T Pag

Witnessed & Understood by me,

Date

12/4/94

Invented by

Recorded by

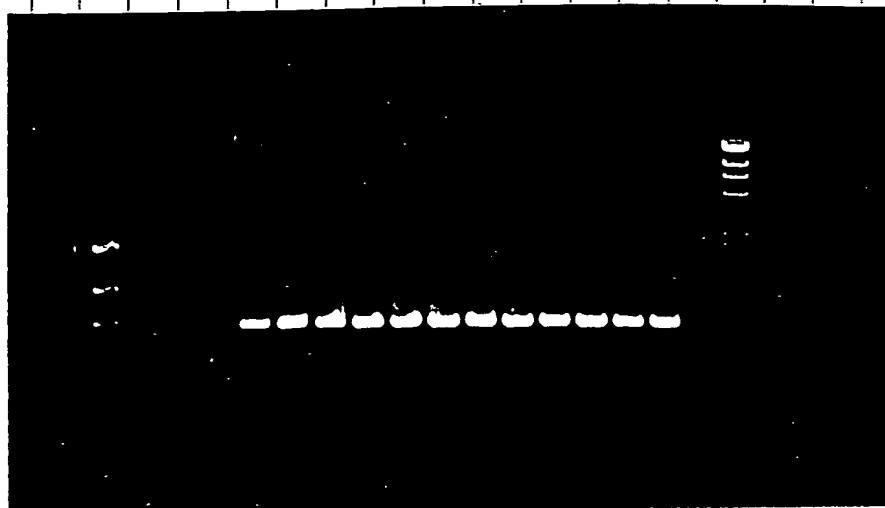
K. Silberman

Date

11/30/94

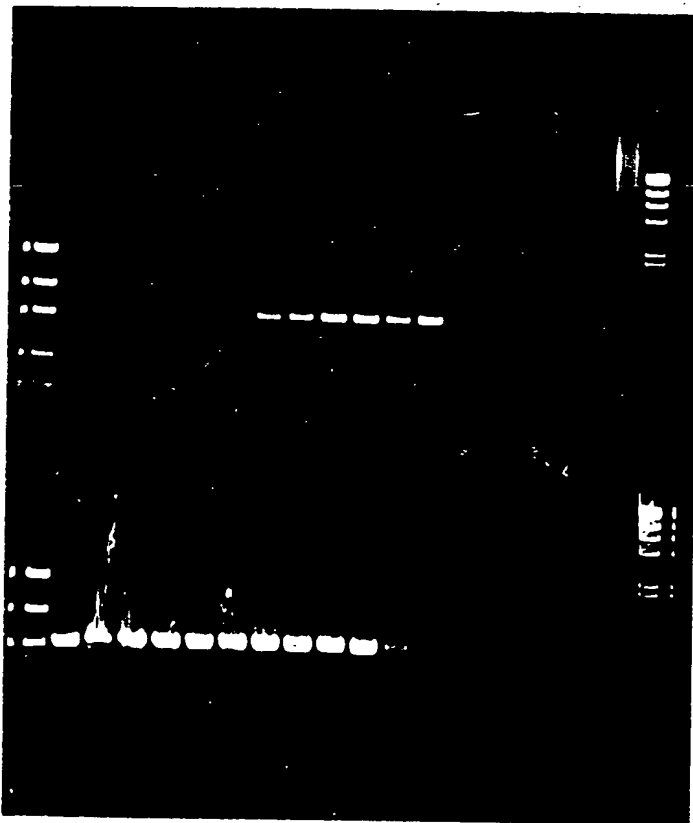
Tag N \_\_\_\_\_

Tag



0 1.5 1 1.5 2 2.5 5

0.001 0.005 0.01 0.05 0.1 0.2 0.5 1 2



10 Tag + 0.001 0.005 0.01 0.05 0.1 0.2 0.5 10 Deepvent

### Result:

- 100 pg + 2.5 cycles seem to be enough
- Deepvent alone with regular primers worked again
- Deepvent at lower concentration works better. With 0.05U good product yield seems seen.
- So may be with earlier run, if the enzyme conc is lower, it might have worked with Deepvent. Try?

T Page No. \_\_\_\_\_

Read & Understood by me,

Date

12/19/94

Invented by

Recorded by

K. Sitarman

Date

12/1/94